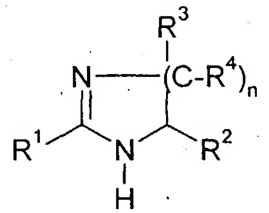
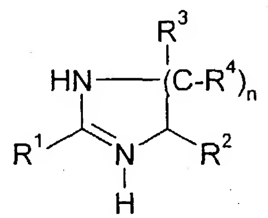
ECTOIN OR ECTOIN DERIVATIVES AND SURFACTANTS

This application is a continuation in part of U.S. application 09/744,945 of January 31, 2001, which is a national phase of PCT/EP99/005239 of July 22, 1999. These two applications, WO 00/07560 and priority documents DE 19834817 of August 1, 1998 and DE 19911775 of March 17, 1999, are each entirely incorporated by reference herein.

An object of the invention is the use of one or more compounds of formulae Ia and Ib



Ia



Ib

the physiologically compatible salts of the compounds of formulae Ia and Ib, and the stereoisomeric forms of the compounds of formulae Ia and Ib, where

R^1	is H or alkyl,
R^2	is H, COOH, COO-alkyl or CO-NH-R^5,
R^3 and R^4	are in each case independently of one another H or OH,
n	is 1, 2 or 3,
alkyl	is an alkyl radical having 1 to 4 carbon atoms, and
R^5	is H, alkyl, an amino acid radical, dipeptide radical or tripeptide radical,

for the preparation of formulations, for example, cosmetic formulations,

for protecting human skin from stress factors, for example, from skin being exposed to and/or having high concentration of surfactants, for example, exogenous surfactants including fatty acid sulfates, especially, sodium laurylsulfate, ammonium laurylsulfate, betaines, especially cocodimethylbetaine and cocoamidopropylbetaine, alkylbenzene sulphonates, fatty acid ether sulfates, especially sodium lauryl ether sulfate, alkylpolyglycosides, especially alkylpolyglucosides and benzalkonium chloride, or salts thereof where such salts exist, and the like, with the proviso that sodium dodecylsulfate is not included.

for protecting human skin from stress factors, in particular from dryness as a result of high temperatures or very low temperatures with low atmospheric humidity and/or from high salt concentration on the skin,

for protecting cells, proteins and/or biomembranes of human skin,

for protecting the microflora of human skin and/or for stabilizing the skin barrier, and

for using the formulations to pretreat the skin, e.g., prevent against all the above-discussed indications or factors, and also

to a method of treating and protecting the skin of a human patient having skin with high exogenous surfactant concentration, wherein the surfactant is a fatty acid sulfate, with the exception of sodium dodecyl sulfate, a betaine, an alkylbenzene sulphonate, a fatty acid ether sulfate, an alkylpolyglycoside, or a salt thereof where such salt exists, comprising

a) administering, for a time sufficient to eliminate said high surfactant concentration, a composition comprising at least one compound of the formulae Ia or Ib or a physiologically compatible salt thereof, or a stereoisomeric form thereof, and

b) thereafter continuing to administer the composition to protect the skin from said high surfactant concentration,

to a method of protecting the skin of a human patient from exogenous high surfactant concentration, comprising: administering, to a patient whose skin is in need of protection from high surfactant concentration, a composition comprising at least one compound of the formulae Ia or Ib, wherein the composition does not contain a surfactant, and wherein said exogenous surfactant is not sodium dodecyl sulfate, and

to a method of protecting the skin of a human patient from high exogenous surfactant concentration, comprising: administering, to a patient whose skin is in need of protection from high surfactant concentration, a composition comprising at least one compound of the formulae Ia or Ib, wherein the composition does not contain said exogenous surfactant and wherein said exogenous surfactant is not sodium dodecylsulfate.

Healthy human skin is colonized on its surface, the Stratum corneum, by a large number of microorganisms which live commensally. From the great diversity of these microorganisms, only a few live continually on the skin and thus form the resident skin flora. The main representatives of the resident flora on the human skin are Staphylococci, Micrococci, Coryneform bacteria and Pityrospora. These live in small colonies on the surface of the Stratum corneum and in the outer epidermis. A second group of microorganisms, which settles temporarily from the outside, in particular on exposed areas of skin, is referred to as transient flora and cannot settle permanently on healthy skin whose microenvironment is heavily determined by the resident microflora. In various regions of the body, the composition of the skin flora varies depending on the microenvironment of the skin. The density of the microorganisms adapts to the respective skin environment so that the ecology of these regions of the body is not unbalanced by excessive settlement by microorganisms. Compared with the normal state of the skin, the number of microorganisms decreases in the case of dry skin, while the number of microorganisms increases in the case of moist skin, for example, as a result of inflammatory changes in the case of eczema, by up to 1000-fold.

Being the barrier layer and surface of the human body, the skin is exposed to a large number of external stress factors. The human skin is an organ which, having diverse specialized cell types - Keratinocytes, Melanocytes, Langerhans cells, Merkel cells and intercalated sensory cells - protects the body against external influences. A differentiation should be made here between external physical, chemical, and biological influences on the human skin. External physical influences include thermal and mechanical influences, and the effect of radiation. External chemical influences are, in particular, the effect of toxins and allergens. External biological influences include the effect of foreign organisms and the metabolic products thereof.

The surface of the human skin is covered by a fatty film which, depending on the given ratios, is to be considered as an oil-in-water or a water-in-oil emulsion and contains numerous active ingredients, such as, for example, enzymes and vitamins, for example, vitamin D. This fatty film, which has been formed from the lipids released from the sebaceous glands and keratinocytes, preserves the moisture of the skin and protects the body as a skin barrier against unfavorable environmental factors. This sensitive equilibrium of the skin barrier is disturbed by external or internal factors.

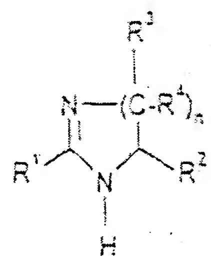
The microorganisms of human skin are subjected to various stress factors. For example, they can be damaged by drying out or by high salt concentrations on the surface of the skin, for example, after perspiring, which can lead to damage of the skin barrier. However, some of these microorganisms - Staphylococci, Micrococci, Corynebacteria and Brevibacteria - usually have the ability to form compatible solutes in order to protect against drying out or high salt concentration and thus contribute to the formation of an intact skin barrier. The compatible solutes, which are also referred to as stress protection substances, are low molecular weight substances in cytoplasm.

Hitherto, it has, for example, been attempted to effect care or protection of human skin by hydrophilic substances which themselves bind water (E.A. Galinski, Experientia 49 (1993) 487-496). However, these hydrophilic substances bind water molecules of the water of hydration as well as free water molecules. Although this leads to a binding of water molecules, it does not lead, for example, to a protection of the hydration sheaths of cells, proteins and cell membranes.

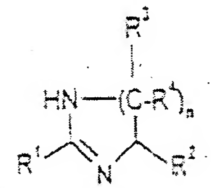
An object of the invention is therefore to provide formulations, for example, cosmetic formulations, the use of which prevent, overcome or at least reduce the abovementioned skin problems and in particular are suitable:

for protecting human skin against stress factors caused by surfactants, with the exception of SDS, for example, against dryness as a result of high temperatures or very low temperatures at low atmospheric humidity and/or against high salt concentration on the skin, for protecting cells, proteins and/or biomembranes of the human skin, for protecting the microflora of the human skin, and/or for stabilizing the skin barrier.

Surprisingly, we have now found that this object is achieved by the use of one or more compounds of formulae Ia and Ib



Ia



Ib,

the physiologically compatible salts of the compounds of formulae Ia and Ib, and the stereoisomeric forms of the compounds of formulae Ia and Ib, where

R^1	is H or alkyl,
R^2	is H, COOH, COO-alkyl or CO-NH-R^5,
R^3 and R^4	are in each case independently of one another H or OH,
n	is 1, 2 or 3,
alkyl	is an alkyl radical having 1 to 4 carbon atoms, and
R^5	is H, alkyl, an amino acid radical, dipeptide radical or

tripeptide radical. These compounds are usually brought into a formulation, for example, a cosmetic formulation.

Within the scope of the present invention, all compounds above and below chosen from the compounds of the formulae Ia and Ib, the physiologically compatible salts of the compounds of formulae Ia and Ib, and the stereoisomeric forms of the compounds of the formulae Ia and Ib are referred to as "ectoin" or "ectoin derivatives."

Ectoin-containing cosmetic formulations protect cells, protein, enzymes, vitamins, DNA, cell membranes and biomembranes of the skin against damage as a result of drying out and the withdrawal of water. The hydration effect of ectoin stabilizes the

water equilibrium of the Stratum corneum and the skin barrier. Ectoin prevents dry and flaky skin.

In addition, ectoin-containing cosmetic formulations protect the microflora of the skin, which is important for an intact skin barrier, against stress as a result of drying out and high ion concentration after perspiring or as a result of exposure to surfactants. The surfactant SDS is not included. The stabilization of the resident skin flora by ectoin or its derivatives is an important prerequisite for the equilibrium of the microenvironment of the skin and the formation of an intact skin barrier.

Ectoin and the ectoin derivatives are low molecular weight cyclic amino acid derivatives which can be obtained from various halophilic microorganisms. Both ectoin and hydroxyectoin have the advantage that they do not react with the cell metabolism.

DE 43 42 560 describes the use of ectoin and ectoin derivatives as moisture-donors in cosmetic products.

US application number 09/744,945 describes the use of ectoin and its beneficial properties against SDS.

The compounds chosen from the compounds of the formulae Ia and Ib, the physiologically compatible salts of the compounds of the formulae Ia and Ib and the stereoisomeric forms of the compounds of the formulae Ia and Ib can be present in the cosmetic preparations as optical isomers, diastereomers, racemates, zwitterions, cations or as a mixture thereof. Of the compounds of formulae Ia and Ib, the physiological compatible salts of the compounds of formulae Ia and Ib and the stereoisomeric forms of the compounds of formulae Ia and Ib, preference is given to those in which R^1 is H or CH_3, R^2 is H or COOH, R^3 and R^4 in each case independently of one another are H or OH and n is 2. Of the compounds of formulae Ia and Ib, the physiologically compatible salts of the compounds of formulae Ia and Ib and, the stereoisomeric forms of the compounds of formulae Ia and Ib, particular preference is given to the compounds (S)-1,4,5,6-tetrahydro-2-methyl-4pyrimidecarboxylic acid (ectoin)

and (S, S)-1,4,5,6tetrahydro-5-hydroxy-2-methyl-4-pyrimidinecarboxylic acid (hydroxyectoin).

The term "amino acid" means the stereoisomeric forms, e.g., D and L forms, the following compounds: alanine, β-alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tryptophan, tyrosine, valine, γ-aminobutyrate, Nε-acetyllysine, Nδ-acetylornithine, Nγ-acetyldiaminobutyrate and Nα-acetyldiaminobutyrate. L-amino acids are preferred. Amino acid radicals are derived from the corresponding amino acids.

The radicals of the following amino acids are preferred: alanine, β-alanine, asparagine, aspartic acid, glutamine, glutamic acid, glycine, serine, threonine, valine, γ-aminobutyrate, Nε-acetyllysine, Nδ-acetylornithine,, Nγ-acetyldiaminobutyrate and Nα-acetyldiaminobutyrate.

The di- and tripeptide radicals are acid amides according to their chemical nature and decompose upon hydrolysis into 2 or 3 amino acids. The amino acids in the di- and tripeptide radicals are bonded together by amide bonds. Preferred di- and tripeptide radicals are built up from the preferred amino acids.

The alkyl groups include the methyl group CH_3, the ethyl group C_2H_5, the propyl groups $CH_2CH_2CH_3$ and $CH(CH_3)_2$ and the butyl groups $CH_2CH_2CH_2CH_3$, $H_3CCHCH_2CH_3$, $CH_2CH(CH_3)_2$ and $C(CH_3)_3$. The preferred alkyl group is the methyl group.

Preferred physiologically compatible salts of the compounds of the formulae Ia and Ib are, for example, alkali metal, alkaline earth metal or ammonium salts, such as Na, K, Mg or Ca salts, and salts derived from the organic bases triethylamine or tris(2-hydroxyethyl)amine. Further preferred physiologically compatible salts of the compounds of the formulae Ia and Ib arise by reaction with inorganic acids, such as hydrochloric acid, sulfuric acid and phosphoric acid, or with organic carboxylic or sulfonic acids, such as acetic acid, citric acid, benzoic acid, maleic acid, fumaric acid, tartaric acid and p-toluenesulfonic acid. Compounds of the formulae Ia and Ib in which

basic and acidic groups such as carboxyl or amino groups are present in equal number form internal salts.

The preparation of the compounds of the formula Ia and Ib is described in the literature (DE 43 42 560). (S)-1,4,5,6-tetrahydro-2-methyl-4-pyrimidinecarboxylic acid and (S,S)-1,4,5,6-tetrahydro-5-hydroxy-2-methyl-4-pyrimidinecarboxylic acid can also be obtained microbiologically (Severin et al., J. Gen. Microb. 138 (1992) 1629-1638). The cosmetic formulation is prepared by converting one or more compounds of formulae Ia and Ib, the physiologically compatible salts of the compounds of formulae Ia and Ib and the stereoisomeric forms of the compounds of formulae Ia and Ib optionally with auxiliaries and/or carriers into a suitable formulation form. The auxiliaries and carriers originate from the group of carriers, preservatives and other customary auxiliaries.

The cosmetic formulations based on one or more compounds of formulae Ia and Ib, the physiologically compatible salts of the compounds of formulae Ia and Ib and the stereoisomeric forms of the compounds of formulae Ia and Ib are applied externally.

Examples of application forms which may be mentioned are: solutions, suspensions, emulsions, pastes, ointments, gels, creams, lotions, powders, soaps, surfactant-containing cleansing preparations, oils and sprays. In addition to one or more compounds of formulae Ia and Ib, the physiologically compatible salts of the compounds of formulae Ia and Ib and the stereoisomeric forms of the compounds of formulae Ia and Ib, any customary carriers, auxiliaries and optionally further active ingredients are added to the formulation.

Preferred auxiliaries originate from the group of preservatives, antioxidants, stabilizers, solubility promoters, vitamins, colorants, odor improvers.

In addition to one or more compounds of formulae Ia and Ib, the physiologically compatible salts of the compounds of formulae Ia and Ib and the stereoisomeric forms of the compounds of formulae Ia and Ib, ointments, pastes, creams and gels can comprise the customary carriers, for example, animal and vegetable fats, waxes, paraffins, starch,

tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silica, talc and zinc oxide or a mixture of these substances.

In addition to one or more compounds of formulae Ia and Ib, the physiologically compatible salts of the compounds of formulae Ia and Ib and the stereoisomeric forms of the compounds of formulae Ia and Ib, powders and sprays can comprise the customary carriers, for example, lactose, talc, silica, aluminum hydroxide, calcium silicate and polyamide powder or a mixture of these substances. Sprays can additionally comprise the customary propellants, for example, chlorofluorocarbons, propane/butane or dimethyl ether.

In addition to one or more compounds of formulae Ia and Ib, the physiologically compatible salts of the compounds of formulae Ia and Ib and the stereoisomeric forms of the compounds of formulae Ia and Ib, solutions and emulsions can comprise the customary carriers, such as solvents, solubility promoters and emulsifiers, for example, water, ethanol, isopropanol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-diethylene glycol butyl ether, oils, in particular, cottonseed oil, groundnut oil, wheatgerm oil, olive oil, castor oil and sesame oil, glycerol fatty acid esters, polyethylene glycols and fatty acid esters of sorbitan or a mixture of these substances.

In addition to one or more compounds of formulae Ia and Ib, the physiologically compatible salts of the compounds of formulae Ia and Ib and the stereoisomeric forms of the compounds of formulae Ia and Ib, suspensions can comprise the customary carriers, such as, liquid diluents, for example, water, ethanol or propylene glycol, suspending agents, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol esters, polyoxyethylene sorbitan esters, microcrystaline cellulose, aluminum metahydroxide, bentonite, agar agar or tragacanth or a mixture of these substances.

In addition to one or more compounds of formulae Ia and Ib, the physiologically compatible salts of the compounds of formulae Ia and Ib and the stereoisomeric forms of the compounds of formulae Ia and Ib, soaps can comprise the customary carriers, such as, alkali metal salts of fatty acids, salts of fatty acid half-esters, fatty acid protein hydrolyzates,

isothionates, lanolin, fatty alcohol, vegetable oils, plant extracts, glycerol, sugars or a mixture of these substances.

In addition to one or more compounds of formulae Ia and Ib, the physiologically compatible salts of the compounds of formulae Ia and Ib and the stereoisomeric forms of the compounds of formulae Ia and Ib, surfactant-containing cleaning products can comprise the customary carrier substances, such as, salts of fatty alcohol sulfates, fatty alcohol ether sulfates, sulfosuccinic half-esters, fatty acid protein hydrolyzates, isothionates, imidazolinium derivatives, methyltaurates, sarcosinates, fatty acid amide ether sulfates, alkylamidobetaines, fatty alcohols, fatty acid glycerides, fatty acid diethanolamides, vegetable and synthetic oils, lanolin derivatives, ethoxylated glycerol fatty acid esters or a mixture of these substances.

In addition to one or more compounds of formulae I a and Ib, the physiologically compatible salts of the compounds of formulae Ia and Ib and the stereoisomeric forms of the compounds of formulae Ia and Ib, face and body oils can comprise the customary carrier substances such as synthetic oils, such as, fatty acid esters, fatty alcohols, silicone oils, natural oils, such as, plant oils and oily plant extracts, paraffin oils, lanolin oils or a mixture of these substances.

Further typical cosmetic application forms are also lipsticks, lipcare sticks, mascara, eyeliner, eyeshadows, blusher, powder, emulsion, wax foundation, sunscreen, and presun and aftersun preparations.

The proportion of the compounds of formulae Ia and Ib, the physiologically compatible salts of the compounds of formulae Ia and Ib and the stereoisomeric forms of the compounds of formulae Ia and Ib in the cosmetic formulation is preferably from 0.0001 to 50% by weight, particularly preferably from 0.001 to 10% by weight, based on the total cosmetic formulation.

Protection of the skin against drying out can be demonstrated, for example, in vivo, for example, by known detection methods such as TEWL (tansepidermal water loss),

corneometry (to determine the moisture in the skin), microtopography (to determine the roughness of the skin) or SELS (surface elevation of living skin).

Ectoin-containing formulations can, for example, protect the skin barrier against the harmful effect of surfactants. The use of a cosmetic ectoin-containing emulsion can significantly reduce the transepidermal water loss typically up to 40% (Fig. 1). Skin pretreated with an ectoin-containing cosmetic formulation is insensitive toward damage of the skin barrier by a surfactant. As a result of the use of an ectoin-containing emulsion, the skin is better protected against surfactant damage of the skin and the water loss associated therewith.

The surfactant is chosen from the group including fatty acid sulfates, especially, sodium laurylsulfate, ammonium laurylsulfate, with the exception of sodium dodecylsulfate, betaines, especially cocodimethylbetaine and cocoamidopropylbetaine, alkylbenzene sulphonates, fatty acid ether sulfates, especially sodium lauryl ether sulfate, alkylpolyglycosides, especially alkylpolyglucosides and benzalkonium chloride, or salts thereof. These surfactants can also be brought into a composition with a compound of formulae Ia or Ib.

An important aim of cosmetics continues to be protection of the skin against stress factors which lead to the drying out of the skin. In particular; dry air during cold or very warm weather conditions leads to a severe loss of water from the skin. Ectoin protects, for example, from a cosmetic O/W emulsion, against drying out (Fig. 2). In addition to the protection against drying out, ectoin-containing cosmetic formulations lead to skin moisture which is significantly better than a corresponding base formulation without ectoin (placebo), but which still comprises 3% glycerol. Furthermore, ectoin-containing cosmetic formulations still effect significantly higher skin moisture compared with the untreated area of skin or areas of skin treated only with the placebo even after 24 hours. Ectoin-containing cosmetic formulations protect the skin against rapid drying out, even against strongly hygroscopic silica gel which is applied directly to the skin. The moisture of the skin can be protected against drying out by the topical application of ectoin-containing formulations, for example, cosmetic formulations over an extended period. Ectoin-containing formulations, for example, cosmetic formulations , are therefore highly suitable for prophylaxis against dry skin.

Stabilization of the biomembranes can be demonstrated, for example, in vitro. Use is made of the fact that propidium iodide is not taken up into the cells if the membrane of the skin cells is intact, and dead cells or cells with a damaged membrane are permeable to propidium iodide and are subject to a red coloration as a result of the absorption of propidium iodide.

By comparing cell cultures which have been pretreated with ectoin prior to damage, for example, by the addition of DMSO, and cells which have not been pretreated, it is possible to ascertain, following subsequent propidium iodide treatment, the effect of the ectoin or its derivatives on the stabilization of the biomembranes.

To determine the cell membrane- and protein-damaging action of surfactants, the red blood cell (RBC) test can, for example, be used. The RBC test is a biological in vitro test for rapid estimation of membrane and protein denaturing properties of surfactants. This test is a measure of surfactant aggressiveness. For a description of this test see Pape et al., Standardization of an in vitro red blood cell test for evaluating the acute cytotoxic potential of tensides, Arzneimitel-Forschung/Drug Research, 40 (I), 4, 498-502 (1990).

In the RBC test erythrocytes are incubated with a surfactant, for example, for a period of 10 minutes. The surfactant destabilizes the membrane of untreated cells such that the cells are partially lysed and their contents such as the hemoglobin are released. The hemoglobin released during cell-wall damage serves as an indicator for the spectrophotometric determination of the membrane damage by a surfactant. By reference to the hemoglobin released, it is possible to determine the number of destroyed erythrocytes.

Ectoin protects the cells against damage by SDS (Fig. 3). The erythrocytes pretreated with ectoin are more resistant toward membrane damage by SDS than untreated cells. The higher the ectoin concentration, the greater the protective effect against membrane damage.

The longer the cells are pretreated with ectoin, the greater the protective effect against membrane damage (Fig. 4) . Stabilization of the cell membranes is both dependent on the

ectoin concentration and on the duration of ectoin pretreatment. The higher the ectoin concentration and the longer the contact time on the erythrocytes, the greater the cell membranes are protected.

Stabilization of the resident microflora can, for example, be demonstrated in vivo. Following ectoin treatment of certain areas of skin, for example, the forearms, the skin is subjected, for example, to dry and/or heat stress in a climatically controlled chamber. The bacteria from the forearms are then isolated, and a "living cell count determination" is carried out, using vital staining and a growth curve for determining the kinetics, for example, by plating out the bacteria on culture plates (plate method) or by the impedance method using conductivity measurements. A comparison of these results with those for areas of skin not pretreated provides evidence of the effect of ectoin or its derivatives on the stabilization of the resident microflora.

Upon further study of the specification and appended claims, further objects and advantages of this invention will become apparent to those skilled in the art.

The examples below serve to illustrate the invention and are in no way to be regarded as a limitation. All percentages are percentages by weight.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

In the foregoing and in the following examples, all temperatures are set forth uncorrected in degrees Celsius and, all parts and percentages are by weight, unless otherwise indicated.

All compounds or components which can be used in cosmetic formulations are either known and available commercially or can be synthesized by known methods.

The INCI names of raw materials used are as follows (the INCI names are by definition given in English):

Raw material	INCI name
Almond oil	Sweet Almond Oil (Prunus Dulcis)
Eutanol G	Octyldodecanol
Luvitol EHO	Cetearyl Octanoate
Oxynex K liquid	PEG-8, Tocopherol, Ascorbyl Palmitate, Ascorbic
Acid,	
	Citric Acid
Panthenol	Panthenol
Karion F liquid	Sorbitol
Sepigel 305	Polyacrylamide, C13-14 Isoparaffin, Laureth-7
Paraffin, low-viscosity	Mineral Oil (Paraffinium Liquidum)
Mirasil CM 5	Cyclomethicone
Arlacel 165	Glyceryl Stearate, PEG-100 Stearate
Germaben II	Propylene Glycol, Diazolidinyl urea, Methylparaben,
	Propylparaben
Bianca perfume	Parfum
Abil WE 09	Polyglyceryl-4 Isostearate, Cetyl Dimethicone
Copolyol,	
	Hexyl Laurate
Jojoba oil	Jojoba Oil (Buxus Chinensis)
Cetiol V	Decyl Oleate
Prisorine IPIS 2021	Isopropyl Isostearate
Castor oil	Castor Oil (Ricinus Communis)
Lunacera M	Cera Microcristallina
Miglyol 812 neutral oil	Caprylic/Capric Triglyceride
Eusolex T-2000	Titanium Dioxide, Alumina, Simethicone

Example 1

The following components are used to prepare a skincare gel (O/W) according to the invention comprising ectoin:

			% by wt.
A	Almond oil	(2)	8.0
	Eutanol G	(3)	2.0

	Luvitol EHO		(4)	6.0
	Oxynex K liquid	(Art. No. 108324)	(1)	0.05
B	Panthenol	(Art. No. 501375)	(1)	0.5
	Carion F liquid	(Art. No. 102993)	(1)	4.0
	Preservative			q.s.
	Water, demineralized			ad 100
C	Sepigel 305		(5)	3.0
D	Ectoin		(1)	1.0

Preservatives which may be used are
0.05% of propyl 4-hydroxybenzoate (Art. No. 107427) or
0.15% of methyl 4-hydroxybenzoate (Art. No. 106757)

Preparation:
The combined phase B is introduced slowly into phase C with stirring. The predissolved phase A is then added. The mixture is stirred until the phases are mixed homogeneously. Phase D is then added and stirred until homogeneous.

Sources of supply: (1) Merck KGaA, Darmstadt, (2) Gustav Heess, Stuttgart, (3) Henkel KGaA, Dusseldorf, (4) BASF AG, Ludwigshafen (5) Seppic, France.

Example 2
The following components are used to prepare a skincare cream (O/W) according to the invention comprising ectoin:

				by wt.
A	Paraffin, low- viscosity	(Art. No. 107174)	(1)	8.0
	Isopropyl myristate	(Art. No. 822102)	(1)	4.0
	Mirasil CM 5		(2)	3.0

	Stearic acid	(1)	3.0
	Arlacel 165	(3)	5.0
B	Glycerol, 87% (Art. No. 104091)	(1)	3.0
	Germaben II	(4)	0.5
	Water, demineralized		ad 100
C	Bianca perfume	(5)	0.3
D	Ectoin	(1)	1.0

Preparation:

Firstly, phases A and B are heated separately to 75°C. Phase A is then added slowly to phase B and stirred until a homogeneous mixture forms. Following homogenization of the emulsion, it is cooled to 30°C with stirring, phases C and D are added, and the mixture is stirred until homogeneous.

Sources of supply: (1) Merck KGaA, Darmstadt, (2) Rhodia, (3) ICI, (4) ISP, (5) Dragoco.

Example 3

The following components are used to prepare a sunscreen lotion (W/O) according to the invention comprising ectoin:

			% by wt.
A	Abil WE 09	(2)	5.0
	Jojoba oil	(3)	6.0
	Cetiol V	(4)	6.0
	Prisorine 2021	(5)	4.5
	Castor oil	(6)	1.0
	Lunacera M	(7)	1.8
	Miglyol 812 neutral Oil	(8)	4.5

B	Eusolex T-2000	(Art. No. 105373)	(1)	3.0
	Glycerol, 87%	(Art. No. 104091)	(1)	2.0
	Sodium chloride	(Art. No. 106400)	(1)	0.4
	Preservative			q.s.
	Water, demineralized			ad 100
C	Perfume		(5)	0.3
D	Ectoin		(1)	1.0

Preservatives which may be used are:
0.05% of propyl 4-hydroxybenzoate (Art. No. 1074.27), or
0.15% of methyl 4-hydroxybenzoate (Art. No. 106757)

Preparation:
Firstly, Eusolex T-2000 is stirred into phase B and heated to 80°C. Phase A is then heated to 75°C and phase B is slowly added with stirring. The mixture is then stirred until homogeneous and then cooled to 30°C with stirring. Phases C and D are then added and the mixture is stirred until. homogeneous.

Sources of supply: (1) Merck KGaA, Darmstadt, (2) Th. Goldschmidt AG, Essen, (3) H. Lamotte, Bremen (4) Henkel KGaA, Dusseldorf, (5) Unichema, Emmerich, (6) Gustav Heess, Stuttgart, (7) H.B. Fuller, Luneburg, (8) Huls Troisdorf AG, Witten.

Example 4

The following components are used to prepare a skincare cream (O/W) comprising ectoin:

				by wt.
A	Paraffin, low-viscosity	(Art.-No. 107174)	(1)	8.0
	Isopropyl myristate	(Art. No. 822102)	(1)	

4.0 Mirasil CM

	5		(2)
	3.0 Stearic acid		(1)
	3.0 Arlacel 165 V		(3)
		5.0	
B	Glycerol, 87% (Art. No. 104091)		(1)
3.0			
	Germaben II		(4)
0.5			
	Water, demineralized		ad
100			
D	Ectoin		
		(1)	x

x = 0 (placebo), 2.5% by weight

Preparation:
Firstly, phases A and B are heated separately to 75°C. Phase A is then added slowly to phase B with stirring and stirred until a homogeneous mixture forms. Following homogenization of the emulsion, it is cooled to 30°C with stirring, phase D is added and the mixture is stirred until homogeneous.

Sources of supply: (1) Merck KGaA, Darmstadt, (2) Rhodia, (3) ICI, (4) ISP.

Example 4a

Using the skincare creams (O/W) comprising ectoin described in Example 4, an in vivo determination of the transepidermal water loss (TEWL) following damage to the skin barrier by SDS treatment is carried out. Firstly, the skin of the subjects (N = 5) on the forearm is treated for one week twice daily with the O/W emulsion (2 mg/cm^2) comprising 2% and 5% of ectoin and an emulsion without ectoin (placebo). In order to increase the TEWL artificially as a result of damage to the horny barrier, the skin is then treated with 80 µL of sodium dodecyl sulfate (SDS; 2% in water) in

an aluminum chamber with occlusion for 24 h. The TEWL determination is carried out in a climatically controlled room at 22°C and an atmospheric humidity of 60% using a TM210 TEWA meter. Fig. 1 shows the TEWL before and after treatment with the ectoin-containing emulsions, and following damage to the skin barrier by SDS. The values for Fig. 1 are given in Tab. 1.

Tab. 1 In vivo determination of the transepidermal water loss (TEWL) following damage to the skin barrier by SDS treatment

	TEWL [$g/m^2/h$]		
	Before treatment	After ectoin treatment for 1 week	After SDS stress
Untreated	5.2	5.6	18
Placebo	5.2	5.8	15.1
2% of ectoin	5.5	5.6	14.2
5% of ectoin	5.1	5.6	10.8

Example 4b

Using the skincare creams (O/W) comprising ectoin described in Example 4, an in vivo determination of the skin moisture following ectoin treatment and dehydration by means of silica gel is carried out. Initially, the skin of the subjects (N = 5) is treated on the forearm for one week twice daily with a cosmetic formulation (2 mg/cm^2) comprising 2% and 5% of ectoin and a formulation without ectoin (placebo). The moisture content of the skin is determined prior to application and after 1 week four hours after the last application. Silica gel 60 (0.2 g/cm^2) is then applied to the test areas of the forearm for two hours with occlusion (dehydration). Following removal of the silica gel, the skin moisture is measured after 10 min, 2 h, 4 h and 24 h in a climatically controlled room at 22°C and an atmospheric humidity of 60%. The results are shown in Fig. 2.

Example 5

Using an aqueous ectoin solution buffered in PBS buffer (22.2 mmol/l of disodium hydrogenphosphate, 5.6 mmol/l of potassium dihydrogenphosphate, 123.3 mmol/l of sodium chloride and 10 mmol/l of glucose), a determination of the membrane-stabilizing action of

ectoin pretreated human erythrocytes against SDS is carried out. The RBC test is used here. The percentage membrane stabilization of cells pretreated with ectoin is determined.

Human erythrocytes ($2x10^8$ cells/ml) are treated for 1 hour with 0%, 0.1%, 0.5%, 1% and 5% of ectoin. The cells are then stressed for 10 min with 0 to 0.04% SDS solution. Then, spectrophotometry is used to determine how many cells have been lysed by reference to the free hemoglobin content. Fig. 3 shows the percentage difference of the lysed cells as a function of the ectoin concentration from the pretreatment compared with an untreated control. The experiment is carried out N = 5 times.

In addition, human erythrocytes ($2x10^8$ cells/ml) are treated with 1% of ectoin for 0 (control), 6, 18 and 24 hours. The cells are then stressed for 10 min with 0 to 0.04% of SDS solution. Then, spectrophotometry is used to determine how many cells have been lysed by reference to the free hemoglobin content. Fig. 4 shows the percentage difference of the lysed cells as a function of the ectoin concentration from the pretreatment compared with an untreated control. The experiment is carried out N = 5 times.

Example 6.

Part a)

Part a) is a pilot study to determine the H_{50} (the concentration of surfactant that produces lysis of 50% of the RBCs in the RBC reagent) values of five surfactants, i.e., sodium lauryl ether sulfate, cocoamidopropylbetaine, alkylpolyglucoside(s), sodium dodecyl sulfate, and benzalkonium chloride, on red blood cells (RBCs) from two human donors.

All experiments used fresh packed RBCs, which showed complete lysis on addition of distilled water, but no autohemolysis.

Procedure:

Washed RBCs were used to prepare serial concentrations to determine the number of cells that, following complete lysis, produce an A_{575nm} of 2.0, equivalent to a concentration of 0.125 mmol/L oxyhemoglobin. That concentration level of packed RBCs was then used as RBC reagent for the subsequent experiments.

25 μL aliquots of RBC reagent were then incubated with various concentrations of surfactants for 10 min to determine H_{50} values.

Surfactant H_{50} was determined either without or after 1 hour or 24 hours of preincubation of RBC reagent at 37°C with gentle agitation.

After 10 minutes, incubation was stopped by centrifugation at 10,000 rpm.

The resulting supernatant, which may have contained hemoglobin, was read in a photometer at 575nm against a blank.

The absorbance read at 100% hemolysis (addition of distilled water) was taken as 100%, reporting all other readings in percent of that value.

The results with 24 hours preincubation produced autohemolysis in some samples. Thus, the main study was conducted with 1 hour preincubation.

Part b)

Part b is the main study. The RBCs were preincubated with a stabilizer prior to addition of surfactants as the lytic agent.

The two stabilizers tested were Ectoin and, as a reference stabilizer, Lecithin. Ectoin was readily soluble in PBS while Lecithin was dispersible. Therefore, concentrations for Lecithin were approximated. The surfactants were all readily soluble in PBS.

Procedure:

1 mL aliquots of BC reagent from two different donors were incubated separately with the following concentrations of the two stabilizers at 37°C with gentle agitation for 1 hr.

- Ectoin: 0.1%, 0.5%, 1%, and 5%,
- Lecithin: 0.1%, 0.5%, 1%, and 5%.

After 1 hour, the preincubated RBCs were incubated for 10 minutes with the pilot study determined H_{50} (after 1 hour's RBC preincubation) of the respective surfactant.

After 10 min., incubation was stopped by centrifugation at 10,000 rpm.

The resulting supernatant was read in the photometer at 575 nm against a blank.

The absorbance read at 100% hemolysis was taken as 100%, reporting all other readings in percent of that value.

The readings (relative to 100% lysis) were calculated in percent of untreated control (protocol with no stabilizer), reporting the percent difference from that control as relative change (increase or decrease) in membrane stability.

The reference stabilizer Lecithin showed no clear membrane-stabilizing effect in either donor. Ectoin showed a dose-response relationship in terms of increased RBC membrane stability in both donors, but with a different extent of stabilization for the two donors. Thus,

the extent of membrane stabilizing activity of ectoin appears to be a function of both concentration and donor.

Figures 5-9 graphically demonstrate the results.

Sources of supply: (Ectoin) Merck KGaA, Darmstadt, (Lecithin) Lucas Meyer (Sodium Laury Ehter Sulfate - Product name: Texapon N SO) Henkel KGaA, Dusseldorf, (Cocoamidopropylbetaine - Product name: Tego Betain L7) Th. Goldschmidt AG, Essen, (Alkylpolyglucosides - Product name: Planlacare 2000 UP) Henkel KGaA, Dusseldorf, (Sodium Docedyl Syulfate - Product name: Bio-Soft D-40) Lepan, (Benzalkonium Chloride Product name: Benzalkonium Chloride Ph Eur, NF) Schuchardt & Co.

The entire disclosure[s] of all applications, patents and publications, cited herein, are incorporated by reference herein.

The preceding examples can be repeated with similar success by substituting the generically or specifically described reactants and/or operating conditions of this invention for those used in the preceding examples.

From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention and, without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.